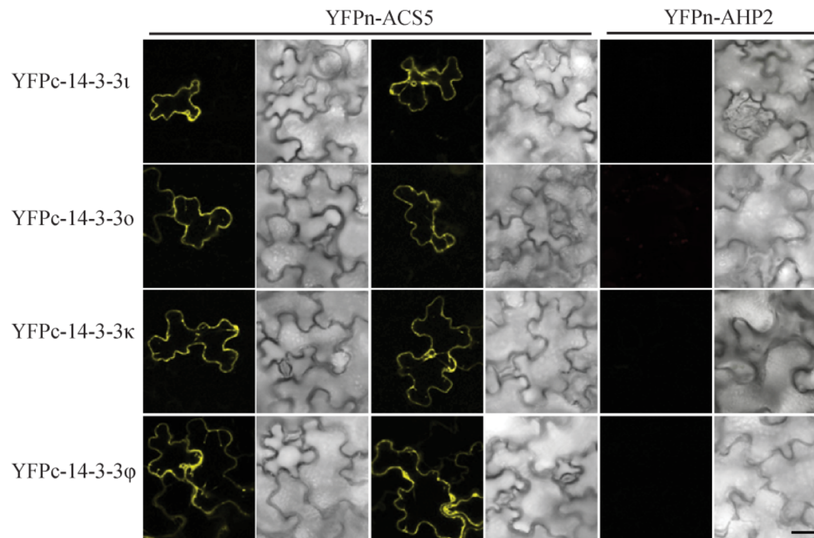
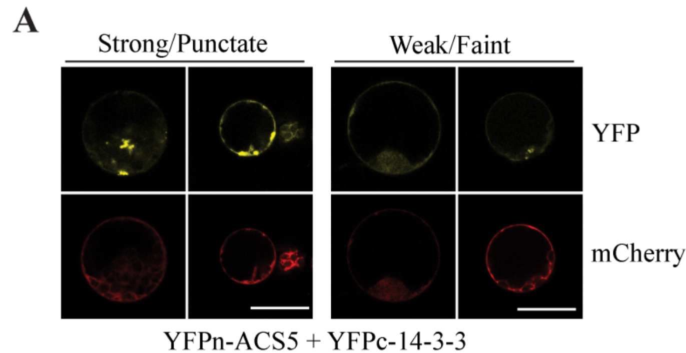
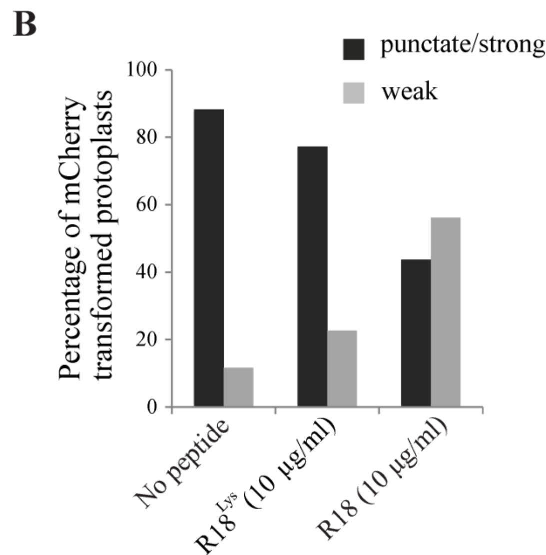
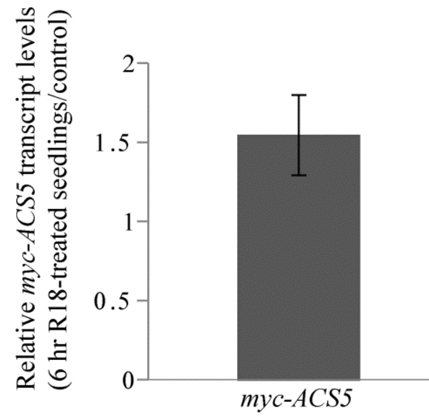


Supplemental Figure 1. ACS5 interacts with multiple isoforms of 14-3-3. Bimolecular fluorescence complementation (BiFC) of YFPc-ACS5 and four different isoforms of 14-3-3 (14-3-3ι, 14-3-3o, 14-3-3κ, 14-3-3φ) fused to YFPn in transiently transformed *Nicotiana benthamiana.* A plasmid expressing a mitochondrial cherry (mCherry) fluorescent marker was used as a transformation marker. Two representative images are shown for each interaction, along with a DIC image of the same cells. YFPn-AHP2 and YFPc-AHP2 were used as negative controls. The YFP signal was observed using confocal microscopy. Scale bar = 50 µm.

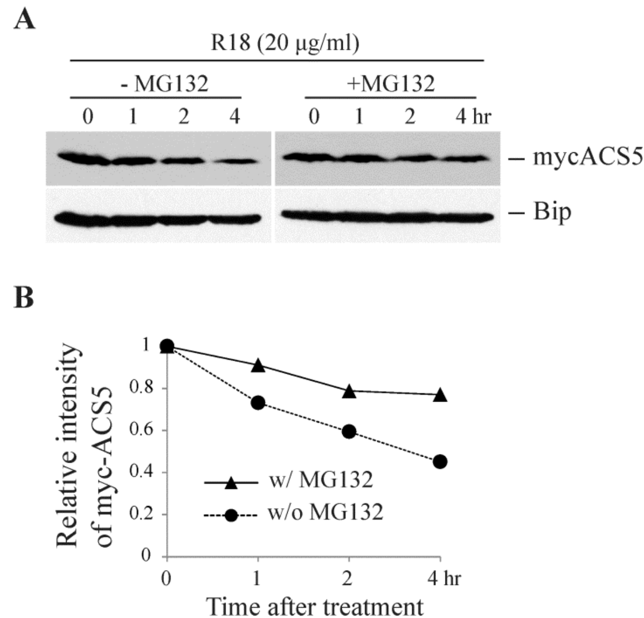




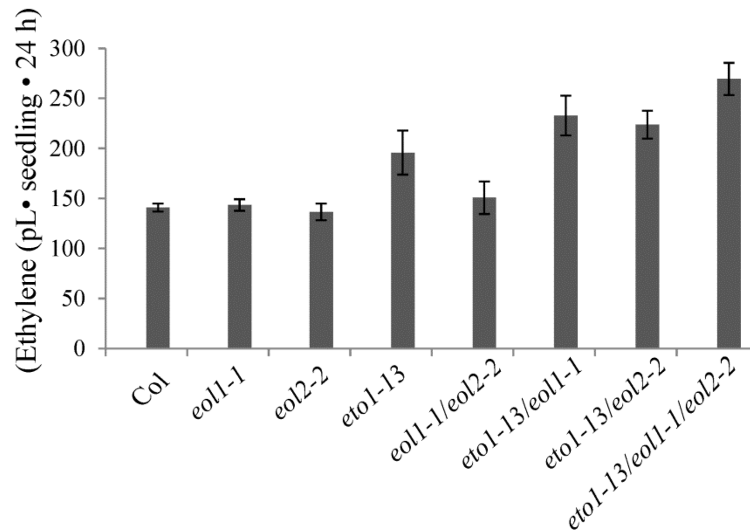
Supplemental Figure 2. ***In vivo*** **interaction between ACS5 and 14-3-3ω in Arabidopsis protoplasts. A.** Classes of positive signals observed in ACS/14-3-3 BiFC interactions. Protoplasts were co-transfected with plasmids expressing YFPn-ACS5, YFPc-14-3-3, and a mCherry fluorescent marker as a transformation marker. Two distinctive classes of BiFC interactions were observed. Most of protoplasts co-transfected with the mCherry marker generated strong, punctate YFP signals (left); a small fraction of the transformed protoplasts exhibited weak, faintly YFP positive signals (right). Scale bar = 20 µm. **B.** Addition of R18 peptide reduces the interaction between ACS5 and 14-3-3 proteins. Protoplasts were co-transfected with plasmids expressing YFPn-ACS5 and YFPc-14-3-3 and were incubated for 12 hours with either R18 or $R18^{Lys}$ peptide (10 µg/ml). Black bars indicate the percentage of protoplasts showing strong/punctate YFP signals; grey bars those showing weak/faint YFP signals. N ≥ 70 per data point.



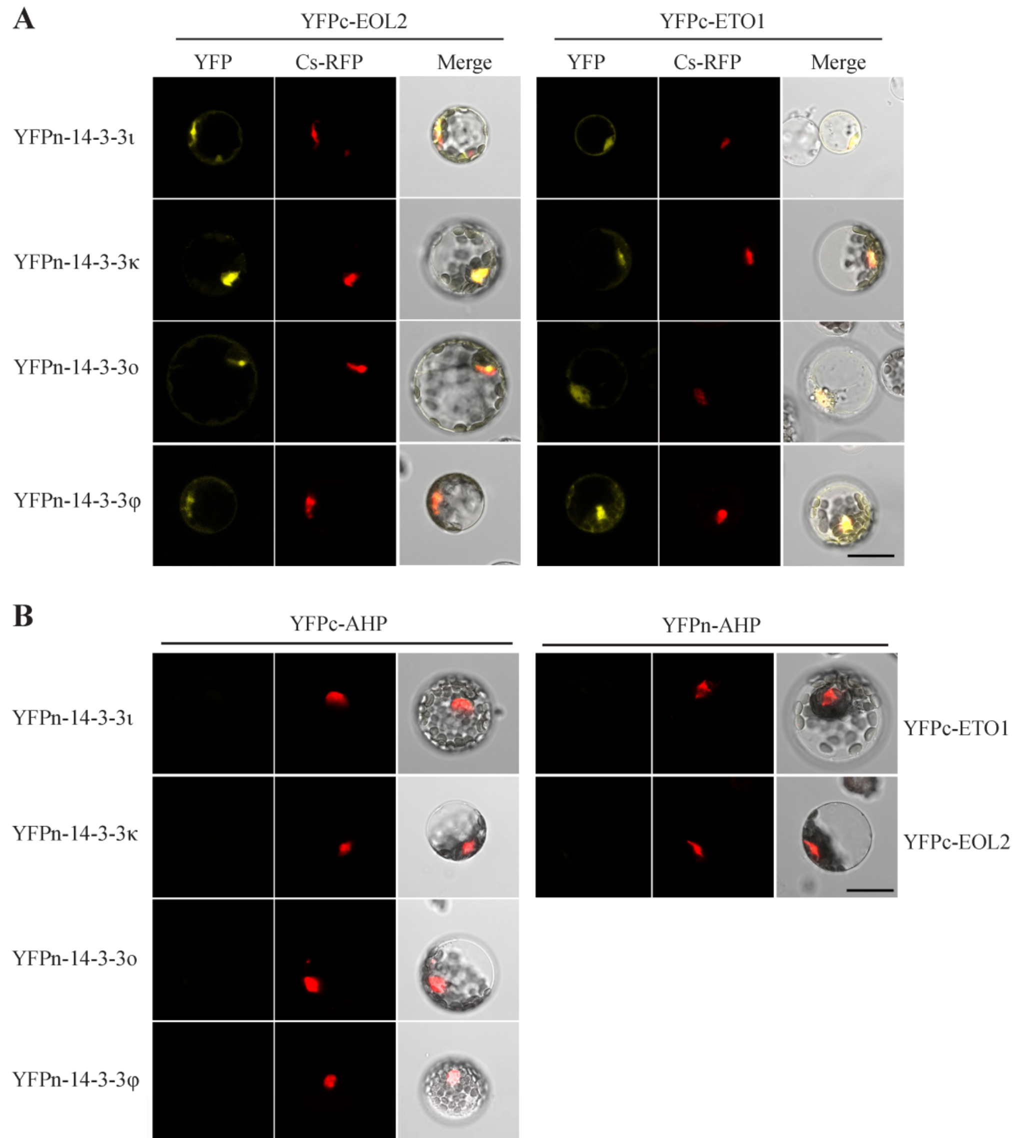
Supplemental Figure 3. Real-time RT-PCR assay of *myc*-ACS5 gene transcript in Arabidopsis seedlings in response to R18 peptide treatment. The bar indicates the level of *myc-ACS5* transcript in the R18 treated sample relative to the non-treated sample. Mean ± SE. Note that the *myc-ACS* transcript level is not decreased in response to R18 treatment.



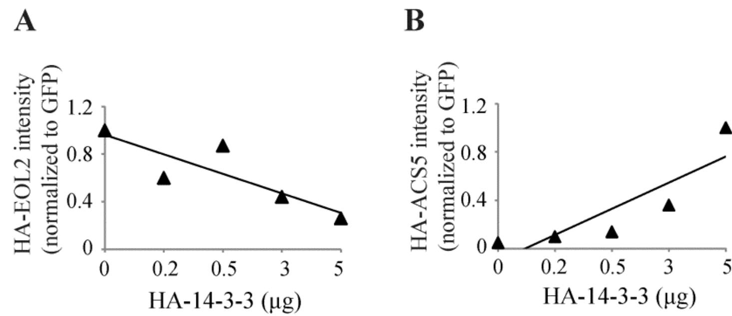
Supplemental Figure 4. R18-mediated destabilization of myc-ACS5 protein is dependent on ubiquitin/26S proteasome activity. A. Dark-grown Arabidopsis seedlings expressing myc-ACS5 protein were treated for various times as indicated with R18 peptide (20 μg/ml) in the presence or absence of MG132 (50 μM). Total protein extracts were used for immunoblotting using either an α-myc or α-BiP (loading control) antibody. **B.** Quantification of the relative intensity of myc-ACS5 bands from the protein blots in **A** using image J software.Intensity of myc-ACS5 bands were normalized to BiP control band intensity, and then these values normalized to the 0 time points, which were set to a value of 1.



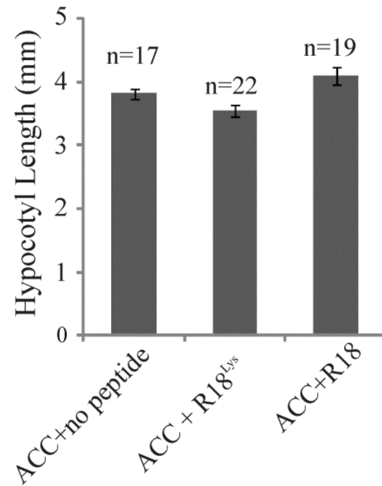
Supplemental Figure 5. Ethylene production of light-grown wild-type and *eto1, eol1* and *eol2* mutant seedlings. The indicated seedlings were grown in light for nine days in GC vials, capped further incubated for 24 h, and then ethylene was measured using gas chromatography as described in the methods. Mean ± SE, N=3.



Supplemental Figure 6. ETO1/EOL2 interacts with multiple isoforms of 14-3-3. Plasmids expressing fusions of YFPc or YFPn to ETO1, EOL2 or four different isoforms of 14-3-3 (14-3-3ι, 14-3-3κ, 14-3-3o, 14-3-3φ) as indicated were transformed into Arabidopsis protoplasts. A plasmid expressing Cs-RFP was used as a transformation and nuclear marker. The protoplasts were imaged for the YFP (first set of panels) or RFP signals (second set of panels) using confocal microscopy. The third panel shows the RFP and YFP signals merged with a DIC image of the protoplasts. Plasmids expressing YFPc-AHP2 or YFPn-AHP2 fusion proteins were used as negative controls. Scale bar = 20 µm.



Supplemental Figure 7. Quantification of the steady-state level of HA-EOL2 and HA-ACS5 proteins with increasing 14-3-3 expression using Image J. The intensity of the HA-EOL2 and HA-ACS5 proteins in Figure 5A normalized to the intensity of the GFP bands in that sample, and then normalized to the value of the 0 (**A**) or 5 μg (**B**) 14-3-3 expressing plasmid data points, which were set to 1.



Supplemental Figure 8. R18 does not affect the hypocotyl length of wild-type seedlings grown in the presence of ACC. Wild-type seedlings were grown for three days in the dark on MS media supplemented with 10 μM ACC in the presence of no peptide, 200 μg/ml R18 or $R18^{Lys}$ peptide and the length of the hypocotyls measured. The mean (of n seedlings as indicated) ± the SE is shown.

Supplemental Table 1. List of primers used in this study

Constructs of entry clones

Genes	Primers	Sequences
14-3-3κ	Kappa-F	CACCATGGCGACGACCTTAAGCAG
	Kappa-R	TCAGGCCTCATCCATCTGCTC
14-3-3φ	Phi-F	CACCATGGCGGCACCACCAG
	Phi-R	TTAGATCTCCTTCTGTTCTTCAGCA
14-3-3ο	Omicron-F	CACCATGGAGAACGAGAGAGCGAAG
	Omicron-R	TTAGATTTTGTTTACCTCATCTTGTTG
14-3-3ι	Iota-F	CACCATGTCATCATCAGGATCCGACA
	Iota-R	TCAGTTCTCAGTGGCATCGGCA
14-3-3ω	Omega-F	CACCATGGCGTCTGGGCGTG
	Omega-R	TCACTGCTGTTCCTCGGTCG
ACS6	ACS6-F	CACCATGGTGGCTTTTGCAACAGAG
	ACS6-R	TTAAGTCTGTGCACGGACTAGCG
ACS7	ACS7-F	CACCATGGGTCTTCCTCTAATGATGGAG
	ACS7-R	TCAAAACCTCCTTCGTCGGT
Ubiquitine3	UBQ3-fwd	CACCATGCAAATCTTTGTCAAGACTCTGACT
	UBQ3-Rev	CTAACCACCTCTGAGACGAAGCAC
RT PCR		
Gene	**Primers**	**Sequences**
myc-ACS5	ACS5-RT-F	CATAGCTGGTTTTCGGCTAGACC
	ACS5-RT-R	ATGAAACAGCTTTCGACAAAAGTG

Supplemental Table 2. List of plasmids used in this study

Constructs	Plasmid type	Source
pENTR-14-3-3κ	ENTRY clone	U10251(ABRC)
pENTR-14-3-3φ	ENTRY clone	U12850(ABRC)
pENTR-14-3-3ο	ENTRY clone	U86636(ABRC)
pENTR-14-3-3ι	ENTRY clone	U60580(ABRC)
pENTR-14-3-3ω	ENTRY clone	U21483(ABRC)
pENTR-ACS5	ENTRY clone	Chae et al., (2003)
pENTR-ACS5^{eto2}	ENTRY clone	Chae et al., (2003)
pENTR-ACS6	ENTRY clone	This study
pENTR-ACS7	ENTRY clone	This study
pENTR-AHP2	ENTRY clone	To et al., (2007)
pENTR-EOL2	ENTRY clone	Christians et al., (2009)
pENTR-ETO1	ENTRY clone	Christians et al., (2009)
pENTR-UBQ3	ENTRY clone	This study
pCL112	Destination vector	Bracha-Drori K, et al. (2004)
pCL113	Destination vector	Bracha-Drori K, et al. (2004)
pEarleyGate 201	Destination vector	Earley et al., (2006)
pEarleyGate 203	Destination vector	Earley et al., (2006)
pEarleyGate 103	Destination vector	Earley et al., (2006)
pEarleyGate 104	Destination vector	Earley et al., (2006)
pTA7002-DEX-GW	Destination vector	This study
pMDC7-GW-myc	Destination vector	This study

Supplemental Figure 3. List of GATEWAY constructs used in this study

Constructs	Entry vector	Destination vector
YFPn-ACS5	pENTR-ACS5	pCL112
YFPn-ACS6	pENTR-ACS6	pCL112
YFPn-ACS7	pENTR-ACS7	pCL112
YFPn-ACS5^{eto2}	pENTR-ACS5^{eto2}	pCL112
YFPn-AHP2	pENTR-AHP2	pCL112
YFPc-AHP2	pENTR-AHP2	pCL113
14-3-3κ-YFPc	pENTR-14-3-3κ	pCL113
14-3-3o-YFPc	pENTR-14-3-3o	pCL113
14-3-3ι-YFPc	pENTR-14-3-3ι	pCL113
14-3-3φ-YFPc	pENTR-14-3-3φ	pCL113
14-3-3ω-YFPc	pENTR-14-3-3ω	pCL113
YFP-14-3-3	pENTR-14-3-3ω	pEarelyGate 104
EOL2-GFP	pENTR-EOL2	pEarleyGate 103
myc-ACS5	pENTR-ACS5	pEarleyGate 203
HA-ACS5	pENTR-ACS5	pEarleyGate 201
HA-14-3-3	pENTR-14-3-3ω	pEarleyGate 201
HA-EOL2	pENTR-EOL2	pEarleyGate 201
HA-ETO1	pENTR-ETO1	pEarleyGate 201
HA-UBQ3	pENTR-UBQ3	pEarleyGate 201
p35S:myc-ACS7	pENTR-ACS7	pTA7002-DEX-GW
p35S:14-3-3-myc	pENTR-14-3-3ω	pMDC7-GW-myc
p35S:HA-EOL2	pENTR-EOL2	pEarleyGate 201

Supplemental Method 1

Quantitative RT-PCR Analysis

Total RNA was extracted from 3-d-old dark-grown *Arabidopsis* seedlings using the RNeasy plus kit (Qiagen). The first-strand cDNA was synthesized from the total RNA using Superscript III reverse transcriptase (Invitrogen) as described by the manufacturer. Quantitative RT-PCR analyses were performed using a SYBR Green premix Ex Taq polymerase (Takara) in a DNA Engine OPTICON 2 (MJ Research) with primers mentioned in Supplemental Table 1. The conditions for PCR amplification were as follows: 94°C for 2 min; 40 cycles of 94°C for 15 s; 58°C for 15 s and 72°C for 15 s. The relative expression for myc-ACS5 in R18 treated seedling expressing myc-ACS5 protein (normalized to b-tubulin as reference gene and to untreated seedling was used to as an internal reference gene) and standard errors were determined using REST 2009 software (Qiagen).

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